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Spatial Genetic Structure among Korean Populations of *Hemerocallis thunbergii* (Liliaceae)

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We investigated the spatial distribution of genotypes among 10 Korean populations of *Hemerocallis thunbergii* Baker (Liliaceae), a herbaceous perennial native to East Asia, using spatial autocorrelation analysis of enzyme polymorphism. A weak genetic structuring exists among the Korean populations. Only 10% (22/225 cases) of Moran's I values for three interpopulational distance classes was significantly different from expected values (E [I] = −0.111). No distinct trend with respect to the distance classes was detected (average Moran's I values were −0.09, −0.12, and −0.11). These results may in part be resulted from a long distance pollen movement by pollinators such as moths and butterflies and then the secondary movement of seeds into adjacent continuously distributed populations in the southwestern Korean Peninsula.

Spatial autocorrelation statistics are summary measures of the dependence of the value of a particular variable at one location on the value of that same variable at other nearby locations. The most commonly used measure is Moran's (1950) autocorrelation coefficient, I, in which geographic neighbors are compared in terms of their deviation from the mean of all observations. Recently, this method of character analysis has been applied to large-scaled structure on a number of plant species because it can provide a detailed picture of geographic variation in characters (e.g., Jensen 1986, Sokal et al. 1986, Chung 1995, 1996).

In Korea, *Hemerocallis thunbergii* Baker grows usually in grasslands and on the margin of forests of the southeastern regions. The lemon yellow, fragrant flowers of *H. thunbergii* are pollinated by moths and butterflies (Hotta et al. 1984, Park and Chung pers. obs.). Usually, moths and butterflies fly a longer interplant distance than do bees (Richards, 1986). In addition, like *H. thunbergii* in Korea, species with more continuously distributed populations would have more chance for gene movement into adjacent populations. Considering these views, it is predicted that the level of gene flow among populations of *H. thunbergii* would be high. As the spatial autocorrelation analysis is useful for understanding genetic structuring among populations (Sokal and Oden 1978a), it may be of interest to determine whether any spatial structure existed among populations of *H. thunbergii*. In this study, we report the spatial structure of allozyme markers among Korean populations of *H. thunbergii*.

Materials and Methods

A total of 466 leaf samples was collected from 10 populations of *H. thunbergii* in Korea (Fig. 1). Sample size for each population was presented in Fig. 1. Leaf samples were cut finely, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton et al. 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. Enzyme extracts were absorbed onto 4×6–mm wicks cut from Whatman 3mm chromatography paper, which were then stored at –70°C until needed. Electrophoresis was performed using 11% starch gels. Fifteen putative loci were resolved from eight enzyme systems using three gel/electrode buffer combinations. Two Poulik buffer systems were used: a modification (Haufler 1985) of Soltis et al. (1983) system 6 was used to resolve leucine aminopeptidase (LAP), fluorescent esterase (FE) and a modification (gel buffer of pH 8.6) of Soltis et al. (1983) resolved diaphorase (DIA), alcohol dehydrogenase (ADH), and β-galactosidase (β-Gal). A morpholine citrate buffer system, a modification (Chung and Kang 1994) of that of Clayton and Tretiak (1972), was used to resolve malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and 6-phosphogluconate dehydrogenase (6PGD). Stain recipes were taken from Soltis et al. (1983), except for the DIA and β-Gal, which were taken from Cheliak and Pitel (1984). Electrophoretic phenotypes were read in the conventional manner with those loci migrating farthest from the origin being designated as 1, the next farthest 2, etc. Within a locus the farthest anodal migrating allele was named a, the next b, etc. Because no controlled crosses were made to analyze segregation of the isozyme banding patterns, all allele and loci designations are "putative" based on subunit structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel (1989). A locus was considered polymorphic if two or more alleles were observed, regardless of their frequencies.

For spatial autocorrelation analysis, mean values were assigned to each population for the mean allele frequency values for alleles at each locus. Because only 10 Korean populations of *Hemerocallis thunbergii* were analyzed in this study, three distance classes were chosen by equalizing sample sizes among the classes. These distance classes were 0<40, 40<90, and 90<193 Km. Every possible pair of populations was considered as a join or a connection and was assigned to one of three distance classes based on the geographic distance between them. Moran's I values were calculated for interpopulational distance classes by

$$I = N \sum_{i} \sum_{j} (W_{ij} Z_i Z_j) \left(\sum_{i} \sum_{j} W_{ij} \sum Z_i^2\right)^{-1}$$

(Sokal and Oden 1978a). N is the number of populations, W_{ij} is the join on weighting matrix, where W_{ij} is set as one if ith and jth population are in the distance class and zero otherwise, $Z_i = \overline{X}_i - \overline{X}$, $Z_j = \overline{X}_j - \overline{X}$ the variables $\overline{X}_i$ and $\overline{X}_j$ are the mean scores for ith and jth population, respectively, and $\overline{X}$ is the mean score for all populations. The value of I ranges between +1 (complete positive autocorrelation, i.e., paired populations have identical values for all characters) and –1 (complete negative autocorrelation). Each I value was used to test significant deviations from the expected values, $E(I)=-1/(N-1)$ (Cliff and Ord 1981). The neighboring populations in the distance class considered, with a significant positive value of Moran's I have similar scores, whereas those with a significant negative value should have different scores. Overall significance of individual correlograms was tested using Bonferroni's criteria (Sakai and Oden 1983). All calculations and statistical analyses were performed using the SAAP program (ver. 4.3) written by D. Wartenberg.

Nei's (1977) G_{ST} values were averaged across polymorphic loci to obtain an estimate of overall population divergence. Two indirect estimates of gene flow were calculated. One estimate of Nm (the number of migrants per generation) was estimated using Wright's (1951) equation: $Nm = (1-G_{ST})/4G_{ST}$. The second estimate was based on the average frequency of private alleles (Slatkin 1985, Barton and Slatkin 1986).

Results

Fifteen putative loci were resolved from eight enzyme systems. *Lap-2*, *Dia-2*, and *Pgd-2* were not included in this study due to faint or inconsistent staining. In addition, *Pgi-1* and *β-gal* loci were monomorphic in all populations. A total of 75 alleles was used for spatial autocorrelation analysis. Only 10% (22/225 cases) of Moran's *I* values for the three interpopulational distance classes was significantly different from the expected value (E[*I*] = –0.111) (Table 1). No distinct trend with respect to the distance classes was detected among Korean populations of *H. thunbergii.* Only nine positive cases were significant from the expected value from distance classes 1 to 2 (0<90 km). In addition, only eight (19%) of 75 alleles of the overall correlogram were significant (Table 1). Again, average Moran's *I* values for the three interpopulational distance classes were very similar: 0.09, –0.12, and –0.11 (Table 1).

Although the mean G_{ST} value was low (0.067), significant differences in allele frequencies among populations were found for all ten loci ($P<0.01$ in each case). The indirect estimate of gene flow based on the mean G_{ST} was high ($Nm = 3.50$) and very similar to the estimate based on private alleles ($Nm = 3.63$, calculated from 0.028 mean frequency of ten private alleles).

Discussion

The ratio of significant *I* values was higher than the intended 5% type I error, suggesting that genetic structuring among populations existed for *Hemerocallis thunbergii.* Only 22 (22/225 cases), however, were significant among populations of *H. thunbergii* and the number of significant overall correlograms was eight. In addition, the average Moran's *I* values for the three interpopulational distance classes (–0.09, –0.12, and –0.11) were almost the same as the expected value (–0.111). These results indicate a somewhat weak population structuring among populations.

In the Korean Peninsula, *H. thunbergii* grows usually in grasslands and on the margin of forests of the southwestern and rarely central regions. The flowers of *H. thunbergii* are fully open in the late afternoon and throughout the night, and close the following morning ("nocturnal habit"). Also, flowers (5–28 per inflorescence) are lemon yellow, fragrant and pollinated by moths and butterflies (Hotta et al. 1984, Park and Chung pers. obs.). A relatively weak genetic structuring among populations ("homogeneity of means in the absence of patterns", Sokal and Oden 1978b) may in part be due to a relatively long distance pollen movement by pollinators such as moths and butterflies. These informations are consistent with the present study of high gene flow among populations ($Nm = 3.50$ and 3.63) and low population differentiation ($G_{ST} = 0.067$). For neutral genes, *Nm* values below 1 indicate that genetic drift is the predominant factor affecting population substructure, whereas *Nm* values above 4 indicate gene flow replaces genetic drift (Hartl and Clark 1989).

It should be noted that, however, since gene movement in plants is a sequential (two step processes via pollen then by seed), observed patch structure will be most influenced by limited seed movement regardless of the distances of pollen movement. In other words, even with long distance pollen movement limited seed movement will result in patches of halfsibs. As *H. thunbergii* has no specialized mechanisms for seed dispersal (many seedlings are found near maternal plants in natural populations), the present results indicate that

Table 1. Spatial autocorrelation coefficients (Moran's *I*) of 75 alleles among Korean populations of *Hemerocallis thunbergii* for the three distance classes (1–3)

Allele	Distance (km)			P^a
	1 (0<40)	2 (40<90)	3 (90<150)	
Lap-1^a	–0.14	–0.17	–0.02	0.689
Lap-1^b	–0.15	–0.20	0.02	0.352
Lap-1^c	–0.02	–0.09	–0.22	0.503
Fe-1^a	–0.02	–0.25	–0.06	0.674
Fe-1^b	0.33**	–0.67**	–0.00	0.003
Fe-1^c	0.19*	–0.30	–0.22	0.122
Fe-1^d	–0.19	–0.06	–0.08	0.942
Fe-1^e	–0.07	–0.33	0.07	0.167
Fe-1^f	–0.23	0.06	–0.16	0.542
Fe-1^g	0.10	–0.36	–0.07	0.249
Fe-2^a	–0.11	–0.19	–0.04	0.825
Fe-2^b	–0.27	0.06	–0.13	0.514
Fe-2^c	0.23*	–0.26	–0.30	0.076
Fe-2^d	–0.17	0.06	–0.22	0.538
Fe-2^e	–0.20	0.04	–0.17	0.618
Fe-2^f	–0.20	0.01	–0.15	0.761
Fe-2^g	–0.16	–0.06	–0.11	1.000
Fe-2^h	–0.27	–0.03	–0.03	0.495
Fe-2^i	–0.20	–0.13	–0.00	0.526
Fe-2^j	–0.11	–0.19	–0.04	0.825
Adh^a	–0.08	–0.19	–0.06	0.864
Adh^b	–0.11	–0.07	–0.15	1.000
Adh^c	–0.04	–0.22	–0.07	0.729
Adh^d	–0.23	–0.10	–0.00	0.556
Adh^e	0.04	–0.18	–0.19	0.566
Adh^f	–0.25	0.04	–0.12	0.611
Adh^g	–0.02	–0.22	–0.09	0.611
Dia-1^a	–0.19	–0.11	–0.04	0.864
Dia-1^b	0.11	–0.42*	–0.02	0.100
Dia-1^c	0.04	–0.24	–0.14	0.556
Dia-1^d	–0.14	0.08	–0.27	0.268
Dia-1^e	–0.21	0.03	–0.16	0.655
Dia-1^f	–0.27	0.01	–0.07	0.471
Dia-1^g	–0.19	–0.11	–0.04	0.864
Mdh-1^a	–0.24	–0.14	0.06	0.276
Mdh-1^b	–0.19	–0.09	–0.06	0.870
Mdh-1^c	–0.23	0.05	–0.15	0.590
Mdh-1^d	0.13	–0.34	–0.12	0.220
Mdh-1^e	0.00	–0.21	–0.12	0.674
Mdh-1^f	–0.21	–0.14	0.02	0.574
Mdh-1^g	0.00	–0.25	–0.08	0.633
Mdh-2^a	–0.12	–0.14	–0.07	1.000
Mdh-2^b	–0.11	–0.19	–0.04	0.825
Mdh-2^c	–0.19	–0.11	–0.04	0.864
Mdh-2^d	–0.32	0.07	–0.08	0.357
Mdh-2^e	–0.07	–0.29	0.02	0.321
Mdh-2^f	–0.24	–0.06	–0.03	0.531
Mdh-2^g	–0.15	–0.01	–0.17	0.902
Mdh-2^h	–0.02	0.05	–0.37*	0.121
Mdh-2^i	–0.11	–0.26	0.04	0.348
Pgd-1^a	0.11*	0.11*	–0.56**	0.029
Pgd-1^b	–0.25	0.12	–0.20	0.328
Pgd-1^c	–0.13	–0.18	–0.03	0.770
Pgd-1^d	–0.22	0.06	–0.17	0.551
Pgd-1^e	0.09	0.11*	–0.53*	0.037
Pgd-1^f	–0.09	–0.03	–0.21	0.605
Pgd-1^g	0.12	–0.01	–0.45**	0.013
Pgi-2^a	0.38**	–0.47*	–0.24	0.006
Pgi-2^b	0.11	0.09	–0.53*	0.035
Pgi-2^c	0.15	–0.25	–0.23	0.176
Pgi-2^d	0.03	0.02	–0.39*	0.055
Pgi-2^e	–0.11	–0.19	–0.04	0.825
Pgi-2^f	0.37**	–0.59**	–0.11	0.011
Pgi-2^g	–0.15	–0.18	–0.01	0.734
Pgi-2^h	–0.34	0.03	–0.02	0.240
Pgi-2^i	–0.12	–0.14	–0.07	1.000
Pgi-2^j	0.05	–0.25	–0.13	0.463
Pgi-3^a	–0.45*	0.12	–0.01	0.074
Pgi-3^b	–0.28	–0.04	–0.02	0.519
Pgi-3^c	0.00	–0.18	–0.16	0.775
Pgi-3^d	–0.03	–0.33	0.02	0.347
Pgi-3^e	–0.49**	0.14*	0.01	0.019
Pgi-3^f	–0.02	–0.38*	0.06	0.113
Pgi-3^g	–0.19	–0.11	–0.04	0.864
Pgi-3^h	–0.13	–0.24	0.04	0.476
Average	–0.09	–0.12	–0.11	

[a] Overall correlogram significance (Bonferroni approximation).

* = $P < 0.05$.

** = $P < 0.01$.

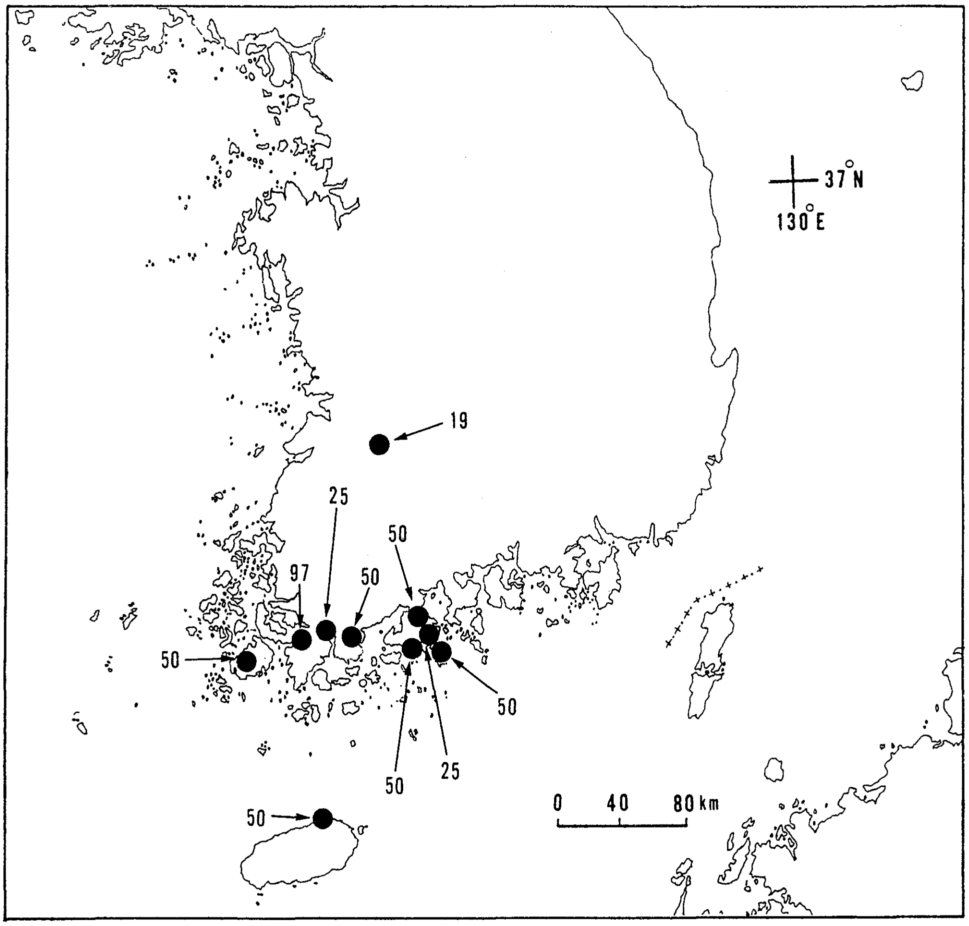

Fig. 1. The location of 10 examined populations of *Hemerocallis thunbergii* in Korea. Sample size is indicated for each population.

the secondary movement of seeds after they have reached the ground is likely and can be extensive (J. L. Hamrick, University of Georgia pers. comm.). Our ongoing study of the fine-scale genetic structure in a population (40×60 m^2 area) of *H. thunbergii*, using 34 alleles as genetic markers, indicated that an approximate minimum genetic patch (individuals having similar genotypes) diameter was 13 m (Kang and Chung unpubl. data). Considering these results, it is suggested that occasional gene movement between continuously distributed adjacent populations in Korea has occurred for a long period of time, which might have been sufficient to retard genetic differentiation among populations.

Finally, a weak structuring of genetic structure within and among populations would in part result from uniformity of environment over the study area ("uniformity of selection", as suggested by Sokal and Oden 1978b). Populations of *H. thunbergii* in Korea commonly grow in open hillsides and grasslands in the southwestern Korean Peninsula. It is suggested that these all factors might have contributed to shaping genetic structure of standing populations of *H. thunbergii* in Korea.

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朴 祺培[a]，鄭 載珉[b]，鄭 明基[b]：韓国におけるユウスゲ集団の遺伝的解析

韓国内のユウスゲ10集団について酵素多型を分析し，遺伝子型のひろがりについて調べたところ，韓国の集団では遺伝的な構造化が弱いことが明らかになった．集団間の距離を3つにクラス分けし，75遺伝子座についてそれぞれのMoran's Iの値を算出したところ，Iの値が期待値をはずれたものはわずかに10％であった．またクラス間の平均のMoran's Iの値にも有意差が認められなかった．このような結果は，韓半島南西部では，蝶や蛾といったポリネーターによって花粉の長距離移動が起こり，さらに種子が二次的な移動によって隣接する集団に動いて行くことにより遺伝的な平均化が起こっていることによるとも考えられる．

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